Brief Articles

Identification of a New Class of Low Molecular Weight Antagonists against the Chemokine Receptor CXCR4 Having the Dipicolylamine−Zinc(II) Complex Structure

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Several low molecular weight nonpeptide compounds having the dipicolylamine−zinc(II) complex structure were identified as potent and selective antagonists of the chemokine receptor CXCR4. These compounds showed strong inhibitory activity against CXCL12 binding to CXCR4, and the top compound exhibited significant anti-HIV activity. Zinc(II)−dipicolylamine unit-containing compounds proved to be useful and attractive lead compounds for chemotherapy of these diseases as nonpeptide CXCR4 antagonists possessing the novel scaffold structure.

Introduction

CXCR4 is a chemokine receptor that transduces signals of its endogenous ligand, CXCL12/stromal cell-derived factor-1 (SDF-1).[1−4] CXCR4 is classified into 7TMGPCR and plays a physiologically critical role by the action of CXCL12 in the migration of progenitors during embryologic development of the cardiovascular, hemopoietic, central nervous systems, etc. In addition, CXCR4 was previously identified as a coreceptor that is used by X4-HIV-1 in its entry into T cells[5] and has recently been proven to be involved in several problematic diseases, including HIV infection, metastasis of several types of cancer,[6−8] leukemia cell progression,[9,10] rheumatoid arthritis (RA).[11,12] Thus, CXCR4 is thought to be a great therapeutic target to overcome these diseases, and several inhibitors directed against CXCR4 have been developed to date.[13−22] We previously found a highly potent CXCR4 antagonist, T140, which is a 14-mer peptide with a disulfide bridge, and its downsized derivative, FC131, which has a cyclic pentapeptide scaffold structure (Table 1).[18−21] Although reduction of the peptide character based on these peptides is underway,[23,24] we would like to discover novel CXCR4 antagonists having nonpeptide structures, since few nonpeptide compounds with low molecular weight have been reported, such as AMD3100 series[14,16] and KRH-1636.[22] Previously, anthracene derivatives having two sets of zinc(II)−2,2′-dipicolylamine (Dpa) complex were identified as the first chemosensors that can selectively bind and sense phosphorylated peptide surfaces.[25] In the present study, we have found several aromatic compounds having the zinc(II)−Dpa structure to be a new class of low molecular weight CXCR4 antagonists.

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Experimental Section

Chemistry. Synthesis of Bis(dipicolylamine)-*p*-xylene−Zn Complexes. Aromatic compounds having the zinc(II)−Dpa structure were previously synthesized as reported elsewhere.[25−28]

For a comparative study, bis(3,3′- and bis(4,4′-dipicolylamine)-*p*-xylenes, **24** and **25**, respectively, were synthesized by treatment of *p*-xylenediamine with the corresponding pyridinecarbaldehydes and sodium triacetoxyborohydride [$NaBH(OAc)_3$] (Figure 1).[29] Zinc(II) complexation in the preparation of **18** and **19** was performed by treatment of bis(dipicolylamine)-*p*-xylenes with NaOH to afford salt-free compounds, followed by addition of aqueous zinc nitrate [$Zn(NO_3)_2$].

Biological Assays. Calcium mobilization,[30] [^{125}I]CXCL12 binding (oil cushion method),[30] and anti-HIV[23] assays were performed as reported previously.

Molecular Modeling Calculations. Molecular modeling calculations were performed using SYBYL program (version 7.0, TRIPOS Inc.). Energy minimizations were performed using Tripos force field. The lowest energy conformation was obtained by random search method.

Biological Results and Discussion

Several aromatic compounds having the zinc(II)−dipicolylamine structure were prepared and surveyed for CXCR4-antagonistic activity based on inhibitory activity against Ca^{2+} mobilization induced by CXCL12 stimulation through CXCR4. The structures and CXCR4-antagonistic activity of these compounds are shown in Tables 1−3. Positive controls **8** (T140), **9** (FC131), and **10** (KRH-1636) showed strong antagonistic activity. Compound **2**, which has two sets of the [bis(pyridin-2-ylmethyl)amino]methylene unit with zinc(II) complexation at the para-position of benzene, showed potent CXCR4-antagonistic activity ($IC_{50} = 0.1\ \mu M$). Compound **1**, which has a piece of this unit, did not show any activity until 1 μM. It suggests that two sets of this unit are required for binding to CXCR4. Compound **3**, which has two sets of this unit at the meta-position of benzene, showed lower activity than compound **2**, suggesting that the presence of this unit at the para-position is critical for

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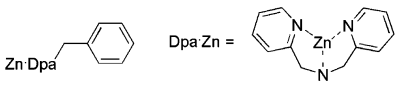
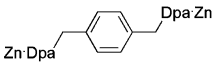
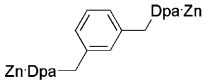
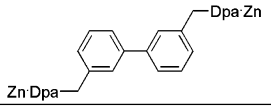
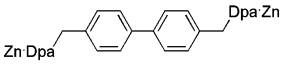
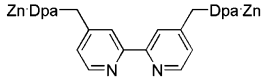
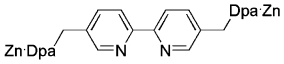
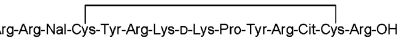
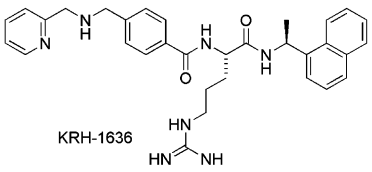
Table 1. Structures and CXCR4-Antagonistic Activity of Aromatic Compounds Having the Zinc(II)−Dipicolylamine Structure (I)

Compd. (No.)	Structure	IC_{50} (μM)[a]
1		> 1
2		0.10
3		0.49
4		0.35
5		0.12
6		0.46
7		0.10
8	 T140	0.0036
9	*cyclo*(-Nal-Gly-D-Tyr-Arg-Arg-) FC131	0.036
10		0.040

[a] IC_{50} values are based on the inhibition against Ca^{2+} mobilization induced by CXCL12 stimulation through CXCR4. All data are the mean values for at least two independent experiments.

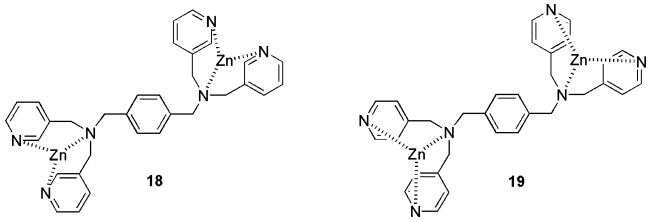


Figure 1. Structures of the zinc(II)−bis(3,3′- and −bis(4,4′-dipicolylamine)-*p*-xylene (**24** and **25**) complexes, **18** and **19**, respectively.

strong CXCR4-antagonistic activity. Biphenyl compounds having two sets of this unit at the 3,3′- and 4,4′-positions, **4** and **5**, respectively, exhibited significantly high antagonistic activity, although the compound with the 4,4′-positions substituted (**5**) is stronger than that having the 3,3′-positions substituted (**4**). It seems to be important that two sets of the [bis(pyridin-2-ylmethyl)amino]methylene unit with zinc(II) complexation are

Table 2. Structures and CXCR4-Antagonistic Activity of Aromatic Compounds Having the Zinc(II)−Dipicolylamine Structure (II)

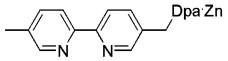
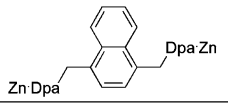
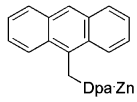
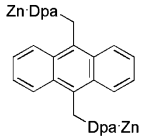
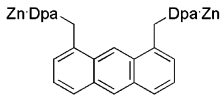
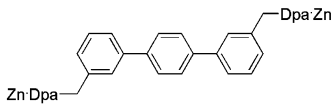
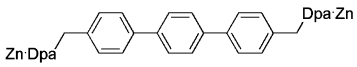
Compd. (No.)	Structure	IC_{50} (μM)
11		> 1
12		0.18
13		0.77
14		0.24
15		0.75
16		> 1
17		> 1

Table 3. Structures and CXCR4-Antagonistic Activity of Aromatic Compounds Having the Zinc(II)−Dipicolylamine Structure and Zinc-Free Compounds

compd	structure	IC_{50} (μM)
18	shown in Figure 1	>10
19	shown in Figure 1	>10
20	zinc-free analogue of **2**	>10
21	zinc-free analogue of **5**	>10
22	zinc-free analogue of **7**	>10
23	zinc-free analogue of **14**	>10
24	zinc-free analogue of **18**	>10
25	zinc-free analogue of **19**	>10

located at 180° of each other as in compounds **2** and **5**, which both have almost the same potency. Furthermore, replacement of the biphenyl unit of compounds **4** and **5** by [2,2′]bipyridinyl did not cause significant change in CXCR4-antagonistic activity, since the [2,2′]bipyridinyl compounds **6** and **7** showed almost the same potency as the biphenyl compounds **4** and **5**, respectively. It may be a matter of course that a [2,2′]bipyridinyl compound having only one [bis(pyridin-2-ylmethyl)amino]-methylene unit with zinc(II) complexation (**11**) did not show any activity until 1 μM, as compound **1** did not. A naphthyl compound (**12**) and an anthracenyl compound (**14**), which have two sets of this unit at the 1,4-positions and at the 9,10-positions,

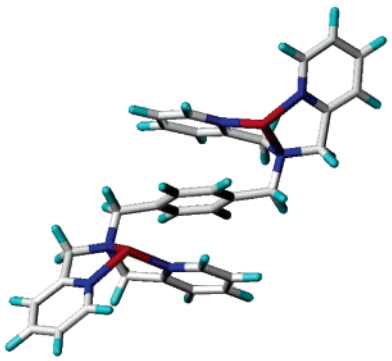


Figure 2. The structure calculated by molecular modeling of compound **2** having the zinc(II)−Dpa structure. Nitrogen atoms, blue; hydrogen atoms, sky blue; zinc atoms, red.

respectively, showed high activity. However, it suggests that the addition of one and two benzene moieties to compound **2** caused a slight decrease in activity. Unexpectedly, an anthracenyl compound having only one [bis(pyridin-2-ylmethyl)-amino]methylene unit with zinc(II) complexation (**13**) exhibited not so high but significant antagonistic activity. It may be reasonable that the anthracenyl compound **15**, which has two sets of this unit at the 1,8-positions, did not show high antagonistic activity, since it is thought to be important that these units are located 180° from each other. In addition, terphenyl compounds **16** and **17** did not show any significant activity until 1 μM, suggesting that it might be unsuitable that two sets of this unit be located far apart from each other.

Next, to investigate whether the location of the nitrogen atom in the pyridine ring of the [bis(pyridin-2-ylmethyl)amino]-methylene unit is critical for expression of CXCR4-antagonistic activity, compounds **18** and **19**, which contained the [bis(pyridin-3-ylmethyl)amino]methylene and [bis(pyridin-4-ylmethyl)amino]methylene units, respectively, with zinc(II) complexation, were synthesized. Neither compound **18** nor **19** showed any significant activity, although antagonistic activity was estimated to 10 μM. This result suggested that the location of the nitrogen atom in the pyridine ring is important either for formation of active conformation or for stable complexation with zinc(II).

Molecular modeling simulation analysis showed that the bis-(3,3′-dipicolylamine) and bis(4,4′-dipicolylamine)-*p*-xylene Zn complexes **18** and **19** did not converge as well as bis(Dpa)−Zn complex **2** did (Figure 2). Values of the coordinate bond lengths between zinc and nitrogen atoms (of the pyridine rings/of the tertiary amine) in the bis(Dpa)−Zn complex **2** are 1.93−1.95 Å, according to molecular modeling calculations. Complex **2** forms a stable conformation, having $\pi-\pi$ stacking among three aromatic rings, as shown in Figure 2. However, values of bond lengths between zinc and nitrogen atoms of the pyridine rings in the complexes **18** and **19** would be ca. 2.9 and ca. 4.2 Å, respectively, which are relatively long. In addition, the proton atoms at the positions 2 and 2′ in the pyridine rings might interfere with the zinc atom to prevent the molecules from forming a stable coordinate conformation.

Furthermore, to verify the indispensability of zinc(II) complexation, zinc-free analogues of **2**, **5**, **7**,and **14**, compounds **20**−**23**, respectively, were assessed for CXCR4-antagonistic activity. Since these zinc-free compounds did not show any significant activity until 10 μM, zinc(II) atoms or conformation constrained by zinc(II) complexation might be indispensable for binding to CXCR4. As a matter of course, zinc-free analogues of **18** and **19**, compounds **24** and **25**, respectively, did not show any significant activity.

Table 4. CXCR4-Binding Activity of Compounds **2**, **5**, **7**, and **12**

compd	IC_{50} (μM)[a]	compd	IC_{50} (μM)[a]
2	0.047	**8** (T140)	0.00093
5	0.18	**9** (FC131)	0.0030
7	0.22	**10** (KRH-1636)	0.034
12	0.42		

[a] IC_{50} values are based on the inhibition of [^{125}I]CXCL12 binding to CXCR4 transfectants of CHO cells. All data are the mean values for at least two independent experiments.

Next, we investigated CXCR4-binding activity of the novel compounds that possess strong CXCR4-antagonistic activity (Table 4). Compounds **2**, **5**, **7**, and **12** showed potent binding activity. Especially, the potency of compound **2** is comparable to that of KRH-1636.

The best and simple compound among the present compounds (**2**) was evaluated for anti-HIV activity. Compound **2** showed significant inhibitory activity against X4-HIV-1-induced cytopathogenicity in MT-4 cells (EC_{50} = 7.1 μM), although anti-HIV activity in cells is lower than CXCR4-antagonistic or -binding activity as usual.[23] Furthermore, the present compounds identified as CXCR4 antagonists showed no significant inhibition (<25%) at 10 μM against Ca^{2+} mobilization induced by MIP-1α stimulation through CCR5 and at 30 μM against Ca^{2+} mobilization induced by sphingosine 1-phosphate stimulation through EDG3 (GPCR).

The present compounds, such as **2**, **5**, **7**, and **12**, have been prepared as binuclear zinc complexes for the use in several assays. The extracellular concentration of zinc is normally ∼100 μg/100 mL (approximately 15 μM): 30% of the total zinc is tightly bound to the metal-binding proteins. The remaining amount (70%) is loosely bound to proteins and easily released from the corresponding proteins.[31] Thus, the extracellular concentration of zinc is sufficiently high for the compounds to be active in vivo. Furthermore, the dipicolylamine (Dpa) unit forms a stable complex with zinc ion (log K = 7.57), indicating that the compounds can maintain an active state as the zinc complex in vivo. The affinity of the Dpa unit for Ca^{2+} and Mg^{2+} ions, which are biologically essential, is considerably low (log K < 3). Thus, it is thought that these ions might not affect the zinc complexes.

Conclusion

The current study presents a new class of nonpeptide CXCR4 antagonists with low molecular weight that have a novel scaffold: a dipicolylamine−zinc(II) complex structure. These compounds showed selective and strong CXCR4-antagonistic activity. These compounds also have basic and aromatic moieties in common with several reported CXCR4 antagonists, e.g., T140, FC131, AMD3100 and KRH-1636, suggesting that these moieties are critical for interaction with CXCR4. The present results provide useful insights for the future design of new CXCR4 antagonists in association with information from other CXCR4 antagonists for development of therapeutic strategies for CXCR4-relevant diseases. Furthermore, anthracene derivatives having two sets of zinc(II)−dipicolylamine, such as compound **14**, might be used as chemical probes to study the biology of CXCR4, as these compounds are used to sense phosphorylated peptide surfaces.

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Supporting Information Available: Additional experimental procedures of novel synthetic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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